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33. Application of the Method of Fermentation-retting.

The Purification of Lac by Fermentation. (Part II)

Hideo Katagiri, Narataro Mugibayashi and *Megumu Omori.*

It was found in the previous paper that nitrogen-impurities in B-seed of lac were remarkably attacked by fermentation with the bacteria Pseudomonas myxogenes sp., isolated by us.

In oder to ascertain what sort of nitrogen was attacked by the fermentation, resins and coloring matters of the B-seed were previously removed by repeated resins and coloring metters of the B-seed were previously removed by repeated alcohol extraction at room temperature until the average yield of the residue attained to 23% by weight.

According to the kinds of B-seed, total nitrogen of the residue above mentioned was found to vary from 3.9 to 5.8% on dry basis. However, any noticeable difference in the proportion of the form of nitrogen was never observed, viz. 5% K_2SO_4 soluble nitrogen including albumin, globulin, proteose, peptone and non-protein, hordein-nitrogen being extracted by hot 80% alcohol, the residual nitrogen (expressed as glutelin-nitrogen) were observed to be 9.3~10.6%, 3.5~3.6% hot alcohol soluble and of 85.9~86.7% of the total nitrogen respectively.

During 4 day fermentation of the sample in Speakman's solution at 35°C, some remarkable decompositions of dry matter, total nitrogen, hordein nitrogen and salt soluble nitrogen were observed to be 25%, 23.5%, 95.2% and 50.0% respectively, while glutelin nitrogen was attacked only 17.7%.

In order to clarify, furthermore, the nature of glutelin nitrogen, extraction of the glutelin fraction was made by 5% NaOH solution. It was found that greater amount (nearly 97%) of glutelin nitrogen dissolved in NaOH, and presence of chitin (nearly 1.5% on dry basis) was ascertained by Proskuriakow's method.

From these results, We would conclude that more advanced purification of B-seed would be capable when more suitable conditions of fermentation were found, since decomposition of glutelin nitrogen attained as high as 45.6% by 7 day fermentation.

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34. On the Protease and Amidase Actions of Bac. Natto.

Yuki Ito.

Casein, gelatin, peptone and protamine were hydrolyzed respectively by autolysate or acetone powder of Bac. Natto between pH 5.0 and 9.0 (the optimum pH 7.5). The hydrolysis value (pH 7.5) was found to be as follows: casein>gelatin>fibrin>

egg albumin>edestin.

The gelatin hydrolysis (pH 7.5) was neither stimuluted nor inhibited by cysteine (0.01–0.002 mol), but was inhibited by metal salts (0.01 mol) in the following order: $AgNO_3$>$CuSO_4$>Hg_2Cl_2>$NiSO_4$>$FeSO_4$. $CO(NO_3)_2$ and $MnSO_4$ seemed to give no influence. The casein or gelatin hydrolysis was not activated particularly by $FeSO_4$, $COCl_2$ and $MnCl_2$ (0.02–0.0002 mol).

Bac. Natto shows di- and tripeptidase activity (hydrosis of diglycine, glycyl-L-leucine, triglycine, leucyldiglycine at pH 6.0 and 7.5). Benzoylglycine, benzoyl-DL-methionine, benzoyl-DL-phenylalanine, benzoyl-DL-leucylglycine, Cl-acetyl-L-leucine and Cl-acetyl-L-phenylalanine were remarkably hydrolyzed, while acetylglycine and acetyl-DL-methionine were not and acetyl-L-phenylalanine slightly at pH 6. The acetone powder also attacked diglycine (pH 7.5–8.0), benzoylglycine (pH 7.5–8.0) and benzoyldiglycine (pH 7.0–7.5). The splitting of diglycine was markedly accelerated by $FeSO_4$ (0.001 mpl), $MnCl_2$ or $COCl_2$, but was inhibited by cysteine (0.0005 mol).

The broth of 2 day culture hydrolyzed proteins, peptone, diglycine and benzoylglycine, but not benzoyldiglycine.

The autolysate and the acetone powder could attack asparagin (pH 7), glutamine and acetamide (pH 7.5–8.0), but benzamide very slightly. L-glutamic acid was deaminated by these enzyme preparations, while L-aspartic acid only by the autolysate.

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35. On the Action of Papain Enzyme. (III)

Masashichi Yoshioka.

It has been found that papain enzyme was activated by sodium formaldehyde sulphoxylate (rongalite, (CH_2OH·OSONa·$2H_2O$)) and a salt of similar sulphur oxyde, (Japan. Pat. 172230) whose activities were conspicluously developed as compared with cystein. (Yoshioka, the Reports of the Institute for Chemical Research, Kyoto Univ. 17, 59 (1949)). This report concerns in the mechanism of the activation by rongalite.

Rongalite has been treated previously with dilute solution of various metallic salt and their influences on digestability of gelatin with papain has been examined respectively by the formoltitration and amino-nitrogen determination.

Further experiment is continued and will be interpreted later.
